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PRODUCTION AND PROPERTIES OF 2,3-BUTANEDIOL

XXII. THE EFFECT OF AEROBIC CONDITIONS ON THE *AEROBACILLUS* FERMENTATION[1]

BY DYSON ROSE[2]

Abstract

In the *A. polymyxa* fermentation, aerobic as compared to anaerobic conditions resulted in an increased yield of acetoin plus diol and a decreased yield of ethanol. As the fermentation proceeded under anaerobic conditions, there was a decrease in the proportion of substrate being used in the formation of acetoin and diol. This decrease was accompanied by an increase in the formation of acetic acid. During fermentation under aerobic conditions, no decrease occurred in the formation of acetoin plus diol.

Introduction

Walpole (10) in 1911 reported a greater yield of butanediol and acetoin from the fermentation of sucrose by *Bacillus lactis aerogenes* in the presence of oxygen than when the fermentation was conducted anaerobically. This observation has since been confirmed with other organisms inducing a similar fermentation, including *Aerobacter faeni* (2) and *Aerobacter aerogenes* (1), and a greater yield of these four-carbon compounds during the fermentation of milk under aerobic conditions has been observed by Prill and Hammer (7) and by other workers. The work of Kluyver *et al.* (5), and of Elion (3, 4), with yeast indicated that an acyloin condensation occurred whenever acetaldehyde accumulated in a fermenting medium, and that such an accumulation could be induced by supplying an alternate hydrogen acceptor. Oxygen was found to be capable of acting in this capacity and it was therefore assumed that the effect of oxygen in increasing the yield of butanediol and acetoin was the result of its action in preventing the reduction of acetaldehyde to ethanol.

In the yeast fermentations, ethanol is normally the only product obtained in large quantities and the above explanation for the increased formation of diol and acetoin under aerobic conditions appears to be satisfactory. However, in the more complex bacterial fermentations it is inadequate. In these bacterial fermentations hydrogen may be released in gaseous form and the

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reduction of acetaldehyde to ethanol need not occur even in the absence of an alternate hydrogen acceptor. It therefore cannot be assumed that the presence of such an acceptor would necessarily increase the total quantity of acetaldehyde accumulated. Furthermore, in these complex fermentations any acetaldehyde formed may be further metabolized in a variety of ways, and even under strictly anaerobic conditions considerable variation in the yields of the various products may occur (8) so that a decreased reduction of acetaldehyde to ethanol need not result in an increased yield of butanediol.

In view of these considerations and the probable importance of aeration in the commercial production of butanediol by means of the *Aerobacillus* fermentation, it seemed desirable to study the effects of oxygen more closely. The results of such studies on fermenting grain mashes have been presented by Adams (1); the present paper presents the results of similar studies using dextrose media.

Materials and Methods

The general procedures used in these studies were those previously described (8, 9). Two locally isolated strains of the organism, N.R.C. Strains C38 (2) and C25, were used. Efficient aeration of the medium was obtained by the use of Kluyver flasks, the purified oxygen or nitrogen being supplied at a rate of about 70 ml. per litre per minute.

The analytical methods have been described in previous papers (6, 8).

TABLE I

COMPARATIVE YIELDS OF THE VARIOUS FERMENTATION PRODUCTS UNDER AEROBIC AND ANAEROBIC CONDITIONS

Product	Yield, moles per 100 moles of dextrose fermented	
	Anaerobic	Aerobic
Butanediol	40.6	Trace (error)?
Acetoin	0.2	51.4
Ethanol	83.2	42.2
Formic acid	2.4	4.1
Acetic acid	6.3	7.4
Lactic acid	2.9	7.0
Succinic acid	1.6	2.5
Malic acid	0.2	1.5
CO_2	200.7	210.3
H_2	97.9	13.5
Acetone*	9.5	?
Carbon recovery	98.3%	92.7%
$\frac{\text{Acetoin + diol}^{**}}{\text{Ethanol}}$	0.96	2.33
Oxidation–reduction index	1.00	2.14

* *The occurrence of acetone under these conditions has been discussed previously; the value given was obtained by calculation (8).*

** *Calculation on a weight basis.*

Yield of Products

Concurrent aerobic and anaerobic experiments were conducted to compare the yields of the various products. The results obtained under anaerobic conditions have been presented in a previous paper (8), and have been shown to be satisfactory by means of a balance sheet. Yield data for a representative aerobic fermentation with Strain C25 are presented in Table I.

These data show the expected increase in the yield of butanediol plus acetoin and also a distinct decrease in the production of ethanol under aerobic conditions. The production of organic acids by this organism is variable and the differences indicated in Table I are not believed to be significant. However, the increase in the production of carbon dioxide is probably a real effect of aeration. Adams (1) did not observe any decrease in the yield of fermentation products due to oxidation, but more thorough aeration was obtained with dextrose solutions than with the viscous grain mashes used by Adams. Carbon dioxide yields from dextrose were considerably in excess of the amount that would be produced by fermentation alone, but the extent to which oxidation occurs appears to be rather limited. The greatly reduced yield of hydrogen in the presence of oxygen indicates that oxygen was acting as a hydrogen acceptor, but the fact that some gaseous hydrogen was formed under aerobic conditions would seem to indicate that no well developed oxidative mechanism is present.

Rate of Formation of Products

Studies on the effects of oxygen on this fermentation were also made on samples withdrawn periodically. The presence of large amounts of dextrose together with the rapidly developing organisms made the determination of dextrose in the earlier samples difficult, and considerable error was thus introduced even though a clarification procedure was used. This error is clearly shown in the 'carbon accounted for' values of Table II where the excessively high values for the first samples are due to the error involved in the determination of dextrose. This error also resulted in a slight upward displacement of the first points of the curves of Fig. 1 (A and B) but did not obscure the shape of these curves.

Since wide variations in the yields of the main products of this fermentation have been observed (8), data are presented for two strains of the organism C38 (2) and C25. The first of these is the more typical in that the butanediol: ethanol ratio, on a weight basis, exceeded 1 : 1 under anaerobic conditions and the acetone yield was low or nil. The second, C25, produced larger quantities of ethanol than of butanediol under anaerobic conditions and may also have produced considerable quantities of acetone.

The yields of fermentation products, other than carbon dioxide and hydrogen, are presented in Tables II and III. Assuming that the fermentation involves the decarboxylation of pyruvic acid, and ignoring lactic and formic acids, a value of 400 moles should be obtained for the 'carbon accounted

TABLE II

THE YIELD OF PRODUCTS, AS MOLES PER 100 MOLES OF DEXTROSE UTILIZED, AT VARIOUS TIMES DURING ANAEROBIC FERMENTATION BY STRAINS C38 (2) AND C25

Sampling time (hr.)	Butanediol	Acetoin	Ethanol	Formic acid	Acetic acid	Lactic acid	Succinic acid	Malic acid	Carbon accounted for*
C38 (2)									
12	60.0	34.4	108.9	59.8	27.6	11.5	4.6	1.1	767.7
24	54.5	14.0	80.5	18.9	6.8	4.3	1.8	0.3	488.8
36	44.5	13.3	76.0	8.3	10.5	3.6	1.7	0.3	431.3
48	44.1	7.1	73.8	2.6	16.6	3.3	1.7	0.1	405.3
72	52.6	4.3	63.3	2.8	21.2	3.2	1.4	0.1	415.0
96	50.4	7.0	60.9	2.9	20.8	2.5	1.0	0.4	409.0
C25									
12	46.7	29.0	169.5	90.0	74.3	17.8	6.6	1.5	966.2
24	21.8	8.8	91.3	16.5	20.8	4.5	2.5	0.7	389.4
36	20.3	6.1	85.3	5.8	20.4	3.7	2.0	0.5	343.9
48	16.6	5.1	98.4	6.3	20.2	3.5	1.7	0.2	348.4
72	18.0	0.8	93.8	0.6	18.9	2.4	1.6	0.4	316.4
96	19.9	1.3	89.6	0.9	18.6	2.5	1.6	1.2	320.8

* *Summation of (yield of product × number of carbon atoms in its molecule).*

TABLE III

THE YIELD OF PRODUCTS, AS MOLES PER 100 MOLES OF DEXTROSE UTILIZED, AT VARIOUS TIMES DURING AEROBIC FERMENTATION BY STRAINS C38 (2) AND C25

Sampling time (hr.)	Butanediol	Acetoin	Ethanol	Formic acid	Acetic acid	Lactic acid	Succinic acid	Malic acid	Carbon accounted for*
C38 (2)									
24	12.1	25.9	26.4	12.6	35.6	10.9	4.6	8.6	374.1
48	33.1	25.9	45.9	24.1	24.5	9.0	2.4	1.0	441.5
72	24.3	34.6	38.2	3.4	9.3	4.9	2.0	1.5	362.7
96	18.9	46.5	40.8	2.5	7.8	5.4	2.3	1.3	391.9
120	0.6	62.9	41.4	3.2	8.3	6.4	2.2	—	384.6**
C25									
24	67.4	11.6	64.5	11.3	3.6	8.7	1.9	0.3	500.4
48	31.2	34.2	63.3	6.3	5.4	5.4	2.0	2.6	439.9
72	4.7	56.3	53.5	1.8	11.2	4.5	2.8	1.3	405.1
96	0	63.0	56.4	1.4	12.1	4.5	2.7	0.3	416.2

* *Summation of (yield of product × number of carbon atoms in its molecule).*
** *Malic acid sample lost.*

for' column. The values obtained under anaerobic conditions, apart from the initial errors in sugar analysis discussed above, are fairly satisfactory for Strain C38 (2) but are too low for Strain C25. This is probably due to the fact that acetone was not determined. Under aerobic conditions these 'carbon accounted for' values are in general quite satisfactory.

In Fig. 1 the yields, in millimoles per litre, of the various products are plotted against the millimoles of dextrose utilized so that the curves obtained indicate the *relative* rates at which each product was produced and also the proportion in which dextrose entered into the various products. In these figures the values for the four-carbon compounds, which are assumed to arise by condensation of two-carbon compounds, have been doubled in order to indicate the true proportionality among the products.

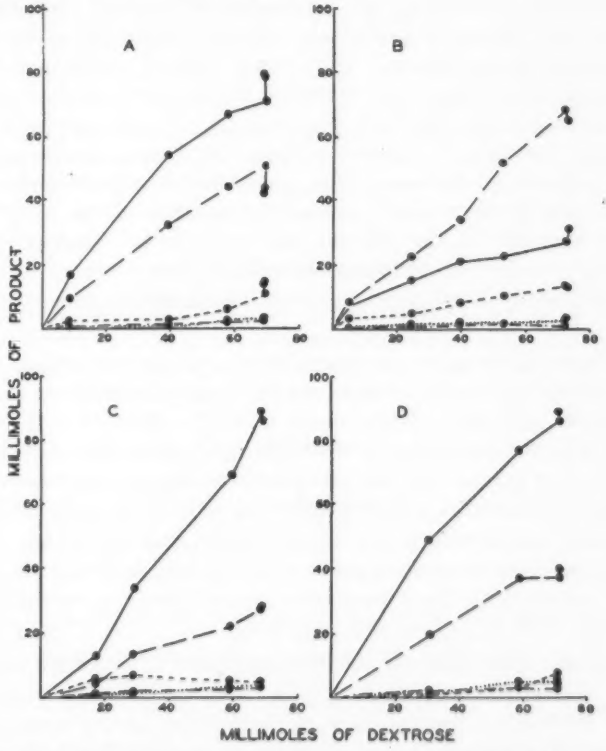

FIG. 1. *Millimoles per litre of product formed plotted against millimoles of dextrose utilized.* ——— *2 (butanediol + acetoin);* — — — *ethanol;* - - - - *acetic acid;* — · — · — *lactic acid;* . . . . . *2 (succinic + malic acid).*

A. C38 (2), anaerobic; analyses at 12, 24, 36, 48, 72, and 96 hr.; total dextrose available, 69.4 millimoles.

B. C25, anaerobic; analyses at 12, 24, 36, 48, 72, and 96 hr.; total dextrose available, 74.4 millimoles.

C. C38 (2), aerobic; analyses at 24, 48, 72, 96, and 120 hr.; total dextrose available, 69.2 millimoles.

D. C25, aerobic, analyses at 24, 48, 72, and 96 hr.; total dextrose available, 71.6 millimoles.

Note.—The yields of four carbon compounds have been multiplied by two in order to indicate the true proportionality in which triose particles (pyruvic acid) enter into the formation of the products.

Two different portions of these curves can be distinguished, the main sloping portions formed while the utilization of dextrose was proceeding and the small vertical sections that represent interconversion of the products after dextrose utilization was complete. These later portions of the curves indicate that small quantities of butanediol may be formed at the expense of ethanol but these interconversions among the products are of no significance in the present discussion.

Under anaerobic conditions the production of ethanol by both strains proceeded at the expense of a relatively constant proportion of the substrate as long as dextrose was present. With Strain C25 the production of ethanol utilized almost one-third of the carbon of the dextrose, an average of 0.92 moles of ethanol being obtained per mole of dextrose fermented. With Strain C38 (2) the yield of ethanol was lower and showed an average value of 0.71 moles per mole of dextrose. The production of butanediol plus acetoin, on the other hand, did not use a constant proportion of the substrate—the proportion decreased as the fermentation progressed. Apparently, during the later stages of the fermentation, more of a common precursor was diverted to the production of another product and less to the production of acetoin and diol.

The yields of succinic plus malic, and of lactic, acids were small and relativel constant, and they cannot account for the substrate diverted from the production of acetoin and diol. With Strain C38 (2) a distinct increase in the proportion of acetic acid formed occurred during the later part of the fermentation, and it is not improbable that this increment of acetic acid was formed at the expense of the acetoin. With Strain C25 none of the products shown in Fig. 1 increased sufficiently to account for the diverted substrate. Probably acetone, the only known product not included in these analyses, was produced to a greater extent late in the fermentation period. The low 'carbon accounted for' values of Table II support this suggestion.

Under aerobic conditions the relative proportion of precursor used in the formation of acetoin and butanediol was much increased, largely at the expense of ethanol. Moreover, each of these major products was produced from a relatively constant proportion of the dextrose utilized; the decline in the production of acetoin plus diol having apparently been prevented by the aerobic conditions. In the fermentation induced by Strain C38 (2) some decrease in the proportion of acetic acid formed is apparent and although the data are not conclusive there appears to have been a compensating increase in the acetoin plus diol yield. With Strain C25 a slight increase in the production of acetic acid occurred late in the fermentation period but this did not markedly detract from the acetoin plus diol yield. As noted above little acetone appeared to be produced under aerobic conditions. With both strains, all of the other acids were produced from relatively small and constant proportions of the substrate.

Conclusions

The data presented show that although in the presence of oxygen a small amount of the substrate may be oxidized to carbon dioxide and water, the main effect of aeration was to alter the proportions of the products. Thus, as frequently noted in the literature, the yield of acetoin plus diol was greater under aerobic conditions, while that of ethanol was lower. As the fermentation proceeded under anaerobic conditions, there was a decline in the formation of acetoin plus diol per mole of dextrose utilized, acetic acid or acetone apparently being formed in their place. This progressive change in the relative amount of substrate being converted to a particular product appears to be an important observation which, to the writer's knowledge, has not been previously recorded. It may presumably be related to some physical or chemical property of the medium or to the reproductive rate of the organism, but further study is required before any adequate explanation can be offered. The effect of oxygen in preventing the decline in butanediol plus acetoin production is also of considerable interest, both from the scientific and the practical point of view.

Acknowledgments

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CHARACTERISTICS OF SOME PSYCHROPHYLIC BACTERIA[1]

By J. J. R. Campbell[2] and G. B. Reed[3]

Abstract

The 10 cultures of bacteria described in this paper developed in an acid (pH 5.0) digest of casein, stored at 4° C. Six of the 10 cultures were *Proteus ichthyosmius*, two *Pseudomonas schuylkilliensis*, one *P. fluorescens*, and one *Lactobacillus leichmanii*. The *Proteus* and *Pseudomonas* grow at 0° C. and the *Lactobacillus* at 0° to 5° C. With the exception of *Pseudomonas* there is no previous record of growth of these organisms at low temperatures.

In connection with another problem, tryptic digests of casein, adjusted to pH 5.0, were stored in carboys, without sterilization, in a cold room at 4° C. (3). The majority remained clear and apparently free from contamination for long periods. An occasional carboy of digest became contaminated. This contamination developed slowly but the initially clear digest became cloudy from the growth in about two weeks. From one such contaminated digest some four species of bacteria were isolated. As a result of the conditions under which they developed and certain unusual properties of the organisms it seemed desirable to record their characteristics.

The organisms were isolated and cultivated in a basal medium composed of 1.0% tryptic digest of casein, 0.5% yeast extract, and 0.5% dipotassium hydrogen phosphate. In experiments where the ability to use a specific substrate was not being tested 0.1% glucose was added to the basal medium. To determine the ability of organisms to grow at temperatures below freezing, agar stabs, 4.0% sodium chloride broth, and 10.0% glycerol broth were used. All three proved satisfactory at −5° C. In determining temperatures of growth the tubes of media were held at incubation temperature for six to seven hours prior to inoculation. In order to determine the optimum pH and the optimum temperature of growth, nephelometer readings were carried out every eight hours. The conditions favouring maximum growth at 24 hr. were considered optimum. In general, the same relative results were obtained at 8, 16, and 24 hr.

Since we were interested in these organisms that could grow under the unusual conditions of pH 5.0 and temperature of 4° C., isolations were made on agar plates at pH 5.0 streaked with the contaminated casein digest and incubated at 4° C. Colonies, which were fully developed at two weeks, were remarkably uniform and gave the impression that only two species were present. However, 10 colonies, which included the slightest variation in colony form, were picked. These isolates were examined in some detail.

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Six of the 10 cultures were identified as a species of *Proteus;* three cultures as three species of *Pseudomonas*, and one culture as a *Lactobacillus.* The somewhat unusual characteristics of the four species are summarized in the following paragraphs.

Proteus sp.

Rods: 0.5 to 0.8 by 1.0 to 2.0 μ, occurring singly. Motile with peritrichous flagella. Gram-negative.
Gelatine stab: Liquefaction.
Agar colonies: Large, white, entire, shiny, raised.
Broth: Slightly floccular with thin pellicle.
Litmus milk: Slight acid production. Rapid peptonization. Litmus reduced.
Indole not formed.
Nitrites produced from nitrates.
H_2S formed.
Acid and gas from dextrose, sucrose, maltose, and mannitol. Lactose not fermented.
Methyl red test negative; Voges-Proskaeur test positive.
Temperature relations: Optimum 20° to 25° C.
Minimum below −5° C.
Maximum 35° to 40° C.
Optimum pH: 6.5 to 8.0. Will initiate growth at pH 5.0.
Salt tolerance: Will grow in up to 9.0% sodium chloride.

This organism appears to be *Proteus ichthyosmius* (Hammer) Bergey *et al.* It differs from the accepted characteristics of the species in the absence of indole formation, in the appearance of colonies on agar, and the absence of fishy odour in milk cultures. However, in view of the very limited amount of work done on *P. ichthyosmius*, it seems more logical to regard the present organism as a member of this species rather than as a new species.

There is no previous record that this species grows at low temperature. Moreover, growth at −5° C. appears to constitute a new low temperature record for the genus *Proteus.*

Pseudomonas schuylkilliensis

Rods: 0.5 to 0.7 by 1.5 to 3.0 μ. Motile with polar flagella. Gram-negative.
Gelatine stab: Liquefaction at one week.
Agar colonies: Flat, opaque, smooth, 1 mm. in diameter.
Broth: Slightly flocculant with light pellicle.
Litmus milk: Slight acid. Digested from the surface down.
Indole not formed.
H_2S not produced.
Nitrates not reduced.
Aerobic.
Pyocyanin produced.

Fluorescent pigment produced.
Temperature relations: Optimum 27° C.
Minimum 0° to 5° C.
Maximum 35° C.
Optimum pH: 7.0. Will initiate growth at pH 5.0.
Salt tolerance: Will grow in up to 6.0% sodium chloride.

Pseudomonas fluorescens

Rods: 0.5 to 0.7 by 1.5 to 2.5 μ. Motile with polar flagella. Gram-negative.
Gelatine stab: Rapid liquefaction.
Agar colonies: Brownish, raised, smooth.
Broth: Heavy sediment with thin pellicle.
Litmus milk: No coagulation. Digestion from the surface down.
Indole not formed.
H_2S not produced.
Nitrites and nitrogen produced from nitrates.
Aerobic.
Pyocyanin not produced.
Fluorescent pigment produced.
Temperature relations: Optimum 25° C.
Minimum below −5° C.
Maximum 37° C.
Optimum pH: 6.8. Will initiate growth at pH 5.0.
Salt tolerance: Will grow in up to 6.0% sodium chloride.

These two pseudomonas forms show the accepted characteristics of *P. fluorescens* and *P. schuylkilliensis*. There are many previous records of low temperature growth of various species of *Pseudomonas*, Hess (2), Thjøtta and Sømme (4). Gibbons (1) isolated *P. fluorescens* from most iced fish fillets examined. Many strains of both species were found to grow rapidly at 0° to 3° C.

Lactobacillus sp.

Rods: 0.3 by 1.0 to 3.0 μ, occurring singly, in pairs, or in short chains. Non-motile. Gram-positive.
Catalase negative.
Agar colonies: Pin-point, white.
Agar slant: Very faint growth.
Broth: Uniformly turbid with heavy growth.
Litmus milk: Completely reduced in 10 hr. No further action. Trace of acid formed in yeast milk.
Gelatine not liquefied.
Nitrites not produced from nitrates.
Acid from dextrose, sucrose, and maltose. Lactose and inulin not fermented. Produces only traces of by-products other than lactic acid from dextrose. Inactive lactic acid formed.

Temperature relations: Optimum 20° to 25° C.
Minimum 0° to 5° C.
Maximum 37° to 40° C.
Optimum pH: 6.5. Will initiate growth at pH 5.0.
Salt tolerance: Will grow in up to 7.0% sodium chloride.

The range of conditions under which this organism grows is most unusual for a lactobacillus. The fact that it rapidly reduces litmus milk without fermenting lactose seems paradoxical; however, when yeast extract is added to litmus milk there is a slight increase in titratable acidity. If this organism is regarded as a non-lactose fermenter it fits into the description of *L. leichmanii* Bergey *et al.* except that the optimum temperature range for growth is much lower than that recorded for *L. leichmanii.*

Discussion

These four species of bacteria, found growing under conditions of unusually low temperature and unusually high acidity, appear to fit into the descriptions of previously known species except for the low optimum and especially the low minimum temperatures at which they grow.

There is no evidence of how these organisms got into the low temperature stored digests. Since the cold room had been used for this purpose for two to three years it is possible that they are the product of variation and acclimatization, from more mesophilic types, to low temperature and high acidity.

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THE STABILITY OF CYANOGEN CHLORIDE[1]

By A. B. Van Cleave[2] and R. L. Eager[3]

Abstract

The stability of cyanogen chloride in contact with Pyrex glass, aluminium, bakelite, brass, and steel at 20° C. has been investigated. The tests indicate that crude or purified cyanogen chloride can be satisfactorily and safely stored in contact with any of these materials except brass, provided that precautions are taken to ensure that only relatively small amounts of water or hydrochloric acid are present. Brass has been shown to be entirely unsatisfactory as a container material for either crude or pure cyanogen chloride as it appears to catalyse a decomposition reaction that is not shown by any of the other materials tested.

Introduction

In the commercial preparation of cyanogen chloride from a cyanide and chlorine, it is probable that small amounts of water, hydrogen cyanide, chlorine, and hydrogen chloride would be present as impurities. Jennings and Scott (1), in discussing the methods of preparation of cyanogen chloride, state that the pure product does not polymerize but that impure products show a varying degree of polymerization, depending upon the conditions. They found this to be especially marked when traces of hydrochloric acid were present. Whitmore (4) states that cyanogen chloride polymerizes on standing to give cyanuric chloride $(CNCl)_3$. It is also known that cyanogen chloride undergoes hydrolysis in alkaline media to produce cyanates but the literature does not contain any direct reference to hydrolysis in acid media.

According to Sartori (2) cyanogen chloride has no corrosive action on iron, lead, aluminium, tin, or silver but it does attack copper and brass slightly, with the formation of a protective coating that prevents further corrosion. No reference to the original literature is given and in view of certain other conflicting information it is desirable to study the storage characteristics of cyanogen chloride in contact with various materials suitable for the manufacture of containers.

Materials

Cyanogen Chloride

Except where noted, crude cyanogen chloride (American Cyanamid Company), which had been filtered through glass wool to remove polymerized materials and solid ammonium chloride, was used. The original liquid was slightly yellow in colour and some yellowish white solid had collected on the walls of the containers. An analysis based on the amount of chloride present after complete hydrolysis by sodium hydroxide indicated the crude liquid to be 99.4% cyanogen chloride.

Some of the crude cyanogen chloride was purified by distilling it from a mixture of cuprous chloride, magnesium oxide, zinc oxide, and calcium

[1] *Manuscript received January 27, 1947.*
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chloride. This material had a freezing point of approximately −7° C. and was water white. Analysis by the above mentioned method gave a mean value for duplicate samples of 100.1% cyanogen chloride.

Metal Samples

Sheet brass and aluminium were used. The steel was band steel. Two coats of bakelite varnish were applied to some of the steel samples so as to ensure that the substrate was entirely covered.

Experimental Method

Since it was desired to measure the pressure increase, if any, the samples were arranged in clean Pyrex glass vessels as indicated in Fig. 1. To facilitate the measuring of pressures from approximately zero to the neighbourhood of four atmospheres, a U-shaped mercury manometer with air enclosed in the sealed off limb was used. The metal samples were cleaned, dried, and weighed and then sealed into the reaction bulbs. Before admitting the cyanogen chloride, the manometers were calibrated by taking readings of known pressures.

About 25 ml. of crude cyanogen chloride was poured into the reaction bulb (which was immersed in a freezing mixture) at *G* (Fig. 1) by allowing it to

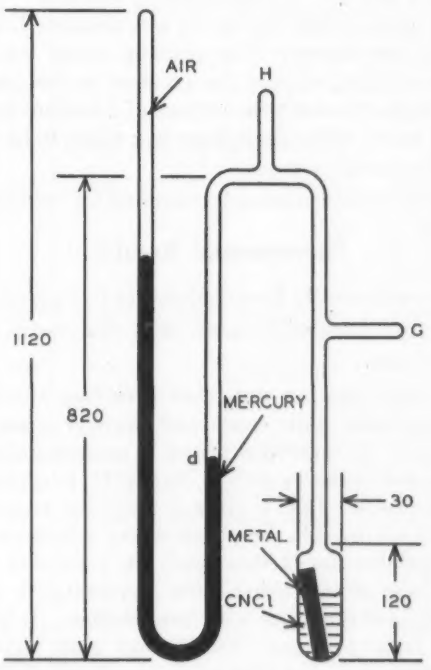

FIG. 1. *Diagram of apparatus used to measure the pressure increase.*

run through a funnel packed with Pyrex glass wool. Following the addition of cyanogen chloride the apparatus was sealed to a vacuum system at *H* (Fig. 1) and evacuated. When evacuated to as low a pressure as possible, the pumps were cut off, the low temperature bath removed, and the cyanogen chloride allowed to melt. When all the solid had melted, it was frozen once more and again evacuated. These operations were intended to remove any inert gases present. The apparatus was sealed off from the vacuum system and placed in a constant temperature bath maintained at $20.0 \pm 0.1°$ C.

When purified cyanogen chloride was being used the above procedure was modified in that the material was distilled into the evacuated apparatus through phosphorus pentoxide.

In order to prevent condensation of liquid cyanogen chloride on the mercury surface at *d* (Fig. 1), which occurred when the room temperature dropped below 20° C., a heater consisting of nichrome wire wound on a strip of transit board was placed along the row of manometers. This heater was shut off for some hours before pressure readings were taken, thus ensuring that the enclosed air in the manometers would be at room temperature when the readings were taken, which was at intervals of approximately one week.

After being in progress for the time indicated in Table I, or Fig. 3, each test was terminated as follows: the reaction vessel was removed from the bath and placed in a dry ice – acetone freezing mixture. When the pressure became steady the glass above *d* (Fig. 1) was thickened, drawn down, and sealed off from the manometer. The reaction vessel was then allowed to come to room temperature, causing the pressure to rise above atmospheric. The tip was then broken beneath the surface of a barium hydroxide solution. The formation of a heavy white precipitate was taken to be a positive test for carbon dioxide in the escaping gases.

The metal strips were then removed, examined for corrosion, and weighed.

Experimental Results

The results may conveniently be considered in two parts:

(*a*) blank tests and tests with metals other than brass, and

(*b*) tests with brass.

(*a*). The blank tests together with tests involving aluminium, steel, and bakelite coated steel with both crude and purified cyanogen chloride are summarized in Table I. In tests Nos. 17 and 18 no manometers were attached; these tests were started as checks on Nos. 10 and 11, in which the manometers were broken by accident. Fig. 2 gives a graphical representation of any pressure change that occurred in those tests where manometers were attached. To avoid undue complication of this figure the results of only one of each duplicate test has been plotted using a few representative pressure readings. In all cases the results of duplicate tests were similar. It is to be noted that in all cases where crude cyanogen chloride was used, including the blanks, some pressure increase was shown over a long period of time. In no case,

TABLE I

SUMMARY OF CHANGES OCCURRING IN THE BLANKS AND TESTS WITH METALS OTHER THAN BRASS

Test No.	Metal	Time in days	Original weight, gm.	Final weight, gm.	Changes occurring in liquid	Change in appearance of metal stripe	Result of CO_2 test
2	Al	580	2.2222	2.2172	Very little change. Original yellow colour had deepened slightly. Very small quantity of grey coloured ppt.	No apparent change	Positive
3	Al	558	2.3581	2.3895	Same as No. 2	No apparent change except that in the vapour space a few fine white crystals had deposited on the edges	Positive
4	Al using purified CNCl	270	2.2040	2.2041	The CNCl remained water white, no sign of polymerization	Same as No. 3	Negative
16	Al using purified CNCl	270	2.2031	2.2031	Same as No. 4	No apparent change	Negative
10	Steel	152	37.3090	37.2425	Final appearance not known definitely as water entered from the bath but there had been no pronounced change		
11	Steel	209	36.1590	36.1410	Same as No. 10		
17	Steel	143	33.1839	33.2018	Same as No. 2	Attacked very slightly, some darkening of the surface. Part of metal in vapour phase mottled yellow	No test made
18	Steel	143	33.5768	33.7043	Same as No. 2	Same as No. 17	No test made
12	Blank (Pyrex glass)	427			Same as No. 2		Positive
15	Blank (Pyrex glass)	551			Same as No. 2		Positive
13	Bakelite	547	40.7974	40.8961	Same as No. 2	Bakelite not attacked. On the surface of the bakelite in contact with the liquid a few small dark coloured specks had formed	Positive
14	Bakelite	398	36.1165	36.2360	Same as No. 2	Same as No. 13	Positive

however, was the pressure increase excessive or likely to lead to an explosion. The metals used in the tests showed very little evidence of corrosion and this is borne out by the fact that only insignificant changes in weight occurred. Small amounts of polymer were present in most of these tests, but certainly most of the original cyanogen chloride was still present when the tests were terminated.

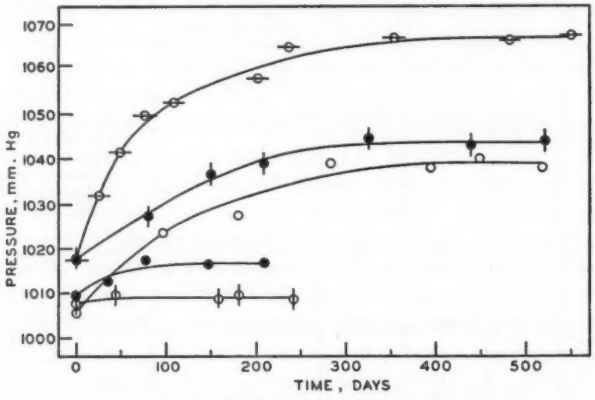

FIG. 2. *The increase of pressure with time.*
-⊖- = *Aluminium, No. 3;* ● = *blank, No. 15;* ○ = *bakelite, No. 13;*
● = *steel, No. 11;* ⊕ = *aluminium, No. 4 with pure cyanogen chloride.*

(*b*). The variation of pressure with time for the tests involving brass is shown in Fig. 3. In test No. 5 (Fig. 3) purified cyanogen chloride was used while in tests Nos. 8 and 9 the crude material was used. In all three cases the liquid darkened in a comparatively short time and a dark brown precipitate settled out. In test No. 9 practically complete solidification occurred in about 250 days. The falling off of the pressure in this case was no doubt due to the complete polymerization or reaction of the cyanogen chloride. Examination of the solid material remaining showed it to consist mainly of a dark brown substance in which was embedded a few white crystals. A small quantity of a dark viscous liquid remained. A few of the white crystals were separated from the mass and tests indicated them to be identical with polymerized cyanogen chloride as obtained by Van Cleave and Mitton (3).

The part of the remaining solid that was soluble in water gave qualitative tests for NH_4^+, Cl^-, and Cu^{++} ions. The bulk of the dark brown material was not identified.

Complete solidification of the cyanogen chloride had not occurred in tests Nos. 5 and 8 up to the time of their termination. The liquid that remained on the termination of these tests was dark coloured and viscous and a con-

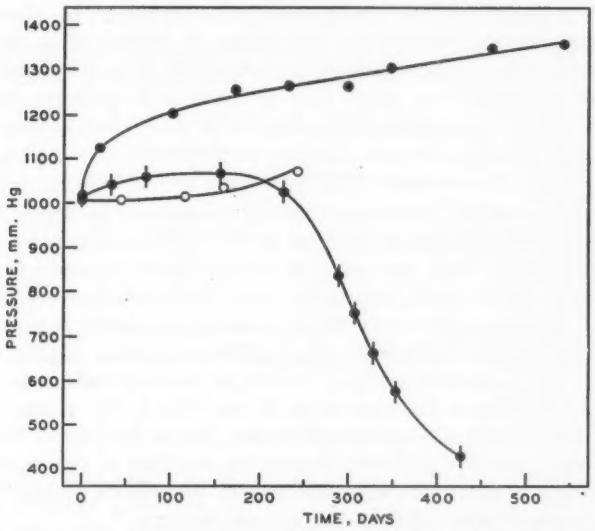

FIG. 3. *The variation of pressure with time for samples in contact with brass.*
● = *No. 8;* ⏚ = *No. 9;* ○ = *No. 5 with pure cyanogen chloride.*

siderable quantity of a dark coloured solid had collected in each tube. In all cases the brass strips showed evidence of extensive corrosion. These tests indicate brass to be entirely unsuitable for use as a container for either crude or pure cyanogen chloride.

Discussion

The results show that a reaction leading to a pressure increase occurred in all cases, including the blanks, where crude cyanogen chloride was used. However, it was only in the case of samples in contact with brass that the pressure reached undesirable proportions at 20° C.

It has been shown (3) that the reaction

$$CNCl + 2H_2O \rightarrow CO_2 + NH_4Cl$$

proceeds readily in the presence of hydrochloric acid. Hence, if the crude cyanogen chloride contained small amounts of water and acid, this would be a likely reaction to occur in these tests and would account for the pressure increases observed. This is supported by the positive tests for carbon dioxide as noted in Table I. It is only necessary to assume about 0.10% of water in the original samples to account for the pressure increases. In all cases, except where brass was used, the pressure has tended towards a constant value after a period of approximately 10 months. After that time there was no significant change in any of the tests involving aluminium, bakelite, steel, or the blanks. Apparently, it took a considerable time for all the water impurity to be used up at 20° C. owing to the low concentration of acid impurities

present. It is likely that this phase of the reaction would be more rapid at elevated temperatures, but there is no reason to believe that the pressure increase would have reached dangerous proportions at any ordinary storage temperature. It should be noted that no increase in pressure occurred in tests Nos. 4 and 16 (aluminium in contact with purified cyanogen chloride) over a period of 270 days nor was there any evidence of polymerization. This seems to bear out the statement that pure cyanogen chloride is quite stable.

The only test in which the pressure increase could not be accounted for on the basis of the above proposed reaction is No. 5 (brass in contact with pure cyanogen chloride). This test showed no significant pressure increase for about 60 days. After that, however, a steady pressure increase was shown and marked decomposition of the liquid cyanogen chloride became evident. It appears that brass catalyses the decomposition of even purified cyanogen chloride with the evolution of a gas. This gas was not definitely identified. The rather large pressure increase shown in test No. 8, Fig. 3, may be partly due to this decomposition reaction. However, the major part of the pressure increase in this case was probably due to the reaction of cyanogen chloride with a few drops of water that were accidentally introduced during the process of sealing the manometer off from the vacuum system.

Conclusions

The tests indicate that crude or purified cyanogen chloride can be satisfactorily and safely stored in contact with Pyrex glass, aluminium, bakelite, or steel at 20° C., provided that precautions are taken to ensure that only relatively small amounts of water and acids, such as hydrochloric acid, are present.

Brass has been shown to be entirely unsatisfactory as a container material for either crude or purified cyanogen chloride as it appears to catalyse some decomposition reaction that is not shown by any of the other materials tested.

Acknowledgment

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DRIED WHOLE EGG POWDER

XXVII. FURTHER OBSERVATIONS ON THE OCCURRENCE OF *SALMONELLA* ORGANISMS IN CANADIAN POWDER[1]

By N. E. Gibbons[2]

Abstract

During 1945, *Salmonella* organisms (other than *S. pullorum*) were isolated from 112 of 400 samples (28.0%) of Canadian dried egg powder. Fourteen types were found: *S. oranienburg, S. typhimurium, S. thompson, S. montevideo, S. newington, S. bareilly, S. manhattan, S. potsdam, S. anatum, S. newport, S. paratyphi B* (tartrate positive), *S. bredeney, S. tennessee,* and *S. selandia* (listed in order of prevalence). *S. pullorum* was isolated from 17 samples. In the majority of samples the most probable number of organisms was less than one per gram. No *Salmonella* organisms were found on the shells of 12,276 commercial eggs; *Salmonella* were isolated from the shells of eight of 24 eggs and later from the meats of two of 144 eggs from infected flocks.

Introduction

Previous studies indicated that *Salmonella* types were present in Canadian dried egg powder produced during 1943 (6). In November, 1944, it was suggested that this survey be resumed. Consequently all plain dried egg powders produced from the latter part of November, 1944, to the end of December, 1945, were examined. By this time four plants were producing dried sugar–egg powder (1, 11) and as most of the others were preparing to produce this type of powder, the survey was discontinued. Since dried sugar–egg powder is used exclusively for baking purposes there should be no health hazard (3, 7).

The present paper summarizes the findings of this study. The result of some incidental work on the source of these organisms is also reported.

Materials and Methods

Composite samples of powder from each carlot of powder produced were sent to a central control laboratory where they were thoroughly mixed under aseptic conditions for bacteriological and chemical analyses (12). Approximately 60-gm. portions of this mixed material were placed in sterile bottles, stored at approximately 40° F., and collected weekly for examination. Only Grade *A* powders were examined.

The methods used have been reported previously (6). Quadruplicate 5 gm. portions were examined throughout the study. From July on, both Difco SS and bismuth sulphite agars were used. Starting in September, whenever the first examination (total 20 gm.) was negative, the remainder of

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the powder (usually 30 to 40 gm.) was cultured. Preliminary identification was made by means of O-sera and fermentation reactions. All cultures identified as *Salmonella* were typed by Dr. P. R. Edwards of the Salmonella Centre, Lexington, Ky.

The most probable number of organisms per gram of powder was determined for a number of samples (6). It was observed that even when three of four 5-gm. lots were positive the M.P.N. was usually less than one per gram. Hence in the latter part of the work, estimates were made only if all the quadruplicate portions were positive.

Results

From the latter part of November, 1944, until Dec. 31, 1945, samples from 400 carlots of powder were examined and *Salmonella* types (other than *S. pullorum*) isolated from 112 (28%). This is five times the incidence reported previously (6). *S. pullorum* was found in 17 samples.

The distribution by plants is shown in Table I. As before, there was no relation between the finding of *Salmonella* organisms and the plate count or total microscopic count of individual carlots, as given in the official reports of the Special Products Board. Rather there were indications of a contamination carried over in the plant. On several occasions, two, three, or even four carlots in succession would contain the same type.

TABLE I

DISTRIBUTION BY PLANTS OF SAMPLES OF POWDER POSITIVE FOR *Salmonella* TYPES OTHER THAN *S. pullorum*, ARRANGED IN ORDER OF PREVALENCE

Plant	Carlots examined	Lots positive	Percentage positive	Average bacterial content thousands/gm.	
				Plate	Microscopic
Western					
I	59	40	67.8	110	2550
H	79	24	30.4	76	720
G	35	9	25.7	74	670
A	13	3	23.1	39	2000
B	62	11	17.7	16	520
K	4	0	0	37	320
	252	87	34.5		
Eastern					
F	27	6	22.2	120	580
C	63	11	17.4	275	1400
E	50	8	16.0	50	2900
D	8	0	0	65	18,350
	148	25	16.8		
Total	400	112	28.0		

If average plate counts over the whole period are taken there is some relation with the incidence of *Salmonella* in the western plants (Table I). There is also some relation with direct microscopic counts. This relation is not as apparent with the eastern plants. Plant *D* operated practically entirely with frozen melange and pasteurized all liquid egg.

There is no apparent relation between the type of drier and the incidence of *Salmonella* organisms. Plants *I* and *H* are very similar in design and the powder is removed rapidly. Plants *B* and *E* are also similar in design and operation, and have about the same incidence. It is also true that the eastern plants *F* and *C* operate at higher temperatures than the western plants. Plant *C* began to use a preheater in March, 1945, and no *Salmonella* organisms were isolated during the period April to December. This was the only preheater used in Canada during the period under review and the results confirm the findings of Schneider (15). However, it is our opinion that the number of contaminated eggs is greater in Western Canada and that this in relation to plant operation determines the incidence and survival of *Salmonella* organisms. This opinion has not been substantiated, as very little experimental work has been done.

The distribution with time is shown in Table II. In this series a few more lots were positive in the fall and winter months than in the summer. This is the opposite of the previous series (6). On the over-all basis, the percentage of positive samples was remarkably constant throughout the year. The few positives in March and April are believed due to laboratory conditions.

TABLE II

DISTRIBUTION OF *Salmonella*-POSITIVE SAMPLES BY MONTHS

Month	From western plants			From eastern plants			Total		
	Carlots examined	Lots positive	Percentage positive	Carlots examined	Lots positive	Percentage positive	Carlots examined	Lots positive	Percentage positive
Nov.	5	1	20.0	3	2	66.6	8	3	37.5
Dec.	19	7	36.8	13	6	46.1	32	13	40.6
Jan.	25	9	36.0	14	4	28.6	39	13	33.3
Feb.	24	9	37.5	15	4	26.6	39	13	33.3
March	25	2	8.0	17	1	5.8	42	3	7.1
April	22	3	13.6	13	0	0	35	3	8.6
May	21	7	33.3	14	2	14.3	35	9	25.7
June	24	8	33.3	10	0	0	34	8	23.5
July	19	4	21.0	11	0	0	30	4	13.3
Aug.	15	8 (10)[1]	53.3 (66.6)	11	0	0	26	8 (10)	30.8 (38.4)
Sept.	14	5 (7)	35.7 (50.0)	7	0	0	21	5 (7)	23.8 (33.3)
Oct.	13	5 (7)	38.4 (53.8)	7	1 (2)	14.3 (28.6)	20	6 (9)	30.0 (45.0)
Nov.	18	6 (8)	33.3 (44.4)	7	1	14.3	25	7 (9)	28.0 (36.0)
Dec.	8	5	62.5	6	3	50.0	14	8	57.1
	252	79 (87)	31.3 (34.5)	148	24 (25)	16.2 (16.8)	400	103 (112)	25.7 (28.0)

[1] *Figures given in parentheses include isolations from larger amounts of powder (see text).*

In Table II is also shown the increase in positive samples due to the examination of the remaining 30 to 40 gm. of powder when the first examination proved negative. From September to December, 85 carlots were examined and *Salmonella* isolated from 36, nine of these or 25% being negative on the first test but positive in the larger sample. These increases made little difference in the order of incidence noted in Table I. Only one additional lot was found positive from the eastern plants (*E*). Four additional isolations were made from powder from Plant *I*, and two each from Plants *H* and *G*. These findings serve to emphasize the spotty distribution of these organisms in egg powder (6). It is also evident that, because of the small samples used in this investigation, the actual percentage incidence is higher than that reported here.

In the eastern plants, the percentage of carlots positive for *Salmonella* was greater in powders prepared from frozen egg than in those from shell or shell and frozen egg mixed (Table III). In one western plant (*H*) the percentage incidence increased when frozen egg was used, but in the others the type of egg used made little difference. In Plant *G* two of the four positive lots from shell egg were obtained with the larger samples of powder. If these are not considered there is little change in the percentage of positive samples from the different types of egg.

TABLE III

CARLOTS OF POWDER, CONTAINING *Salmonella* ORGANISMS, PRODUCED FROM SHELL EGGS, MIXTURES OF SHELL AND FROZEN EGG, AND FROZEN EGG

Plant	Shell			Shell and frozen			Frozen		
	No. carlots	No. positive	Percentage positive	No. carlots	No. positive	Percentage positive	No. carlots	No. positive	Percentage positive
Western									
A	4	1	25	1	—	—	8	2	25
B	16	3	18	33	5	15	13	3	23
G	10	4	40	4	1	25	21	4	19
H	13	2	15	38	8	21	28	14	50
I	22	14	63	30	21	70	7	5	71
K	—	—	—	—	—	—	4	—	—
	65	24	37	106	35	33	81	28	34
Eastern									
C	7	—	—	53	11	20	3	—	—
D	1	—	—	1	—	—	6	—	—
E	18	—	—	25	4	8	7	4	57
F	7	2	29	15	2	13	5	2	40
	33	2	6	94	17	18	21	6	29
Total	98	26	26	200	52	26	102	34	33

Fifteen types of *Salmonella* were found. These are listed in their order of incidence in Table IV. Most of the types found previously (6) were again encountered. *S. oranienburg* was the most common type, although 27 of the 45 strains were isolated from the powder produced by one plant. The three

TABLE IV

TYPES OF *Salmonella* ISOLATED AND THEIR DISTRIBUTION

Type	No. of samples positive in:		Total
	252 western lots	148 eastern lots	
S. oranienburg	45	2	47
S. typhimurium	14	5	49
S. pullorum	12	5	17
S. thompson	9	5	14
S. montevideo	0	9	9
S. newington	7	1	8
S. bareilly	4	1	5
S. manhattan	4	—	4
S. potsdam	3	—	3
S. anatum	2	1	3
S. newport	—	1	1
S. paratyphi B	1	—	1
S. bredeney	1	—	1
S. tennessee	1	—	1
S. selandia	1	—	1
			134

strains of *S. potsdam* were from the same plant (*H*) from which it was previously isolated (6). Of the new types, *S. montevideo* was found only in powder from eastern driers; eight of the nine isolations were from one drying plant. The strain of *S. paratyphi B* was tartrate positive; this is apparently a rare type in fowl. As far as can be determined, *S. selandia* has not been reported previously from fowl or eggs. It might be noted that one culture of *Aerobacter aerogenes* having antigens VI and VII was encountered from Plant *I*. Recently, several reports listing types of *Salmonella* isolated from dried egg have appeared elsewhere (14, 15, 18).

In eight lots of powder two types of *Salmonella* were encountered. In all but one instance the types were of different groups since it was impossible to distinguish types in the same group with the sera available for the preliminary screening. The following pairs were obtained: *S. oranienburg* and *S. bareilly; S. oranienburg* and *S. newington; S. typhimurium* and *S. manhattan; S. typhimurium* and *S. bareilly; S. newington* and *S. bareilly. S. pullorum* was also encountered, along with *S. oranienburg* and with *S. bredeney*. All of the above were isolated from the product of one drier (*I*). *S. pullorum* and *S. typhimurium* were isolated from a sample from Plant *H*.

Of the 17 strains of *S. pullorum* isolated, 14 were found only on bismuth sulphite agar plates. The superiority of this medium over SS agar for *S. pullorum* was also noted in isolations from chicks. This has recently been

reported by others (16). Schneider (15) claims that selenite F enrichment is superior to tetrathionate broth for the isolation of *S. pullorum*, although it is not stated whether equally good results were obtained with both the SS and desoxycholate citrate agars used. In the present study, of 51 isolations of types other than *S. pullorum*, 36 were isolated on both media, nine from SS agar only, and six from bismuth sulphite agar only. When organisms of the same group were found on both media it was assumed they were of the same type.

Of the 17 strains of *S. pullorum* isolated, 12 were checked for the 'X' variant (2, 19) by means of partially absorbed sera. Three were classed as the variant, five as intermediates, and four as the normal strain.

Estimates of the most probable number of *Salmonella* present were made on 54 lots. During most of the period, estimates were made only on samples in which three or four of the quadruplicate lots were positive. Of the 54 samples checked, in all but four the M.P.N. was less than one per gram. In four samples the M.P.N. was 1.3, 2, 3, and 9.4 per gram.

During June and July, samples from 12 carlots of dried sugar–egg powder from Plant *F*, six from Plant *A*, and three from Plant *B* were examined. *S. pullorum* was isolated from one lot from Plant *A*. No other *Salmonella* types were encountered in this product.

Source of *Salmonella* Infection

There is a growing literature to support the suggestion made previously (6) that in hens' eggs the *Salmonella* organisms, other than *S. pullorum*, come from fecal contamination on the shells, as has been noted by many authors with other types of fowl. Watt (17) isolated *S. montevideo* from the meats of eggs responsible for an outbreak, and in an examination of over 5000 hen eggs recovered *S. cholerasuis* and *S. derby* from a few. He apparently did not find *Salmonella* on the shells. Although the evidence was not very definite, the outbreak reported by Crowe (5) was probably due to infected meats. On the other hand, it has been shown that the shells of eggs laid by hens carrying *Salmonella* organisms are at times contaminated with these organisms (8). Gordon and Buxton (10) also indicate that in an outbreak in a hatchery the organisms were probably carried by fecal contamination. Chase and Wright (4) failed to isolate any *Salmonella* types, other than *S. pullorum*, from the meats of 2000 and the exterior of 400 eggs. A recent contribution (16) gives ample evidence that more dirty eggs carry *Salmonella* organisms than do clean eggs. Our attempts to trace the source of these organisms are reported below.

From November, 1944, to August, 1946, eggs were received from six points across Canada at monthly intervals for another study (13). Five dozen each of Grades *A* large, *A* medium, *A* pullet, *B*, and *C* were received each time and broken out for drying. The shells of each lot were collected in sterile beakers, packed loosely, and covered with tetrathionate broth. In all, the

shells of 195 lots or 975 dozen eggs were examined. Samples (approximately 100 ml.) of the broken out egg were also examined. *S. pullorum* was isolated from two samples of melange and one lot of shell. No other *Salmonella* organisms were encountered.

In addition, the shells and contents of some 48 dozen other eggs of various grades have been examined with negative results. This represents a total of 12,276 eggs from various parts of Canada. However, this is a very small sample when it is considered that between 120,000 and 180,000 dozen eggs go into one carlot of powder.

In an infected flock one might expect different results. When a number of chicks from a Saskatchewan hatchery were found to be infected with *S. bareilly*, the eggs from which the chicks were hatched were traced back to two farms, and in May, 1945, a dozen eggs from each flock was obtained. Both shells and egg meats were examined by a technique already described (9). The untreated egg is placed between ring clamps, which are kept painted with tincture of iodine, and the meat is removed as previously indicated. The shell is then broken up using two pairs of flamed forceps and put into tetrathionate broth. The small amounts of iodine carried over are neutralized by the excess thiosulphate in the broth. *S. bareilly* was isolated from the shells of two of the eggs from one farm (*L*) and of six of the eggs from the other (*M*). No organisms were found in the meats. In August six dozen eggs were obtained from each of these farms. All from one lot (*L*) were negative, but *S. newington* was found in the meats of two eggs from the other farm (*M*). No *Salmonella* organisms were recovered from the shells of these eggs.

In Plant *I* in May, 1946, the shell and contents of 40 dirty eggs were examined as well as swabs of the shells of 89 dirty eggs. No *Salmonella* was obtained. Samples of melange, shell drip, etc., were taken at various times but only one isolation was made; *S. pullorum* was found on an emulsifier screen. Three samples collected on one day in July from the by-pass to the high pressure pump and one from the holding vat were positive for *S. oranienburg*.

At Plant *H*, 52 dirty eggs were examined, with negative results. Five lots of shells from at least three dozen eggs each were collected at the breaking tables and cultured but no *Salmonella* was found. However, *Salmonella* types (*S. bareilly*, *S. oranienburg*, and *S. thompson*) were recovered in 7 out of 12 samples of the albumen draining from the barrels of egg shells. During the same period, isolations were made from 4 of 20 samples of melange taken at various points in the plant (*S. potsdam*, *S. bareilly*, *S. thompson*). It cannot be said whether these organisms came from the inside or outside of the eggs, although the greater incidence in the shell drippings would point to the latter.

It would seem therefore that where flocks are known to be carriers or if large enough samples of commercial eggs are examined, *Salmonella* organisms may be recovered from the eggs. The evidence presented is still insufficient to say that it is due solely to fecal contamination.

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QUANTITATIVE STUDY OF THE HILSCH HEAT SEPARATOR[1]

By A. F. Johnson[2]

Abstract

The 'heat separator' is a very simple device for attaining a temperature drop of the order of 30 centigrade degrees by means of a stream of air. An air jet enters a long cylindrical chamber near one end, and is directed so as to maintain a high velocity vortex. Air drawn off from the centre of the vortex is cooled, while the remainder, flowing down the length of the chamber, is warmed. The present paper describes results of a quantitative study of the performance of the device. Although the maximum efficiency is only about 15%, as compared with an ideal adiabatic expansion of the total flow, it provides a practical means of cooling for many purposes.

Introduction

The apparatus described in this paper as a 'heat separator' is a simple device for producing cold by the separation of a high velocity gas vortex into cold and warm streams. Compressed air enters a long cylindrical chamber through a tangential nozzle near one end. If that end of the cylinder is closed by a diaphragm that permits the escape of the gas only from the central region, while the other end is throttled, the gas escaping through the central diaphragm is cooled, while the gas escaping through the other end is warmed. With our apparatus, temperature differences in excess of 40 centigrade degrees have been obtained between the inlet and the cooled gas stream.

The device was introduced to this continent by R. M. Milton, who described it informally at a meeting of the American Chemical Society in April 1946 (2). He had obtained it from Hilsch's laboratory in Erlangen. No data on its performance were available, and, since it seemed probable that it would have useful applications, the present quantitative study was undertaken. Since the submission of the present paper, a paper by Hilsch (1) has appeared. The results of the present investigation are in substantial agreement with those reported by Hilsch and are given below in summary only.

The apparatus (Fig. 1) consists of a cylindrical block, B, of lucite containing a central cylindrical chamber, C. Air under pressure enters this chamber tangentially through the fine orifice, A (0.047 in. in diameter). The entering stream is guided into a spiral flow, with a high angular velocity, by a C-shaped eccentric ring, which is shown at E, and at xy in the cross section, $I - I'$. The chamber is extended to the right by means of a German silver tube, T_1, whose internal radius is equal to that of the eccentric at y. On the left is a small orifice, O, on the axis of the chamber leading to another tube, T_2. The fitting F_2 projects into the chamber so that the opening of the orifice O is in the plane of the near side of the eccentric.

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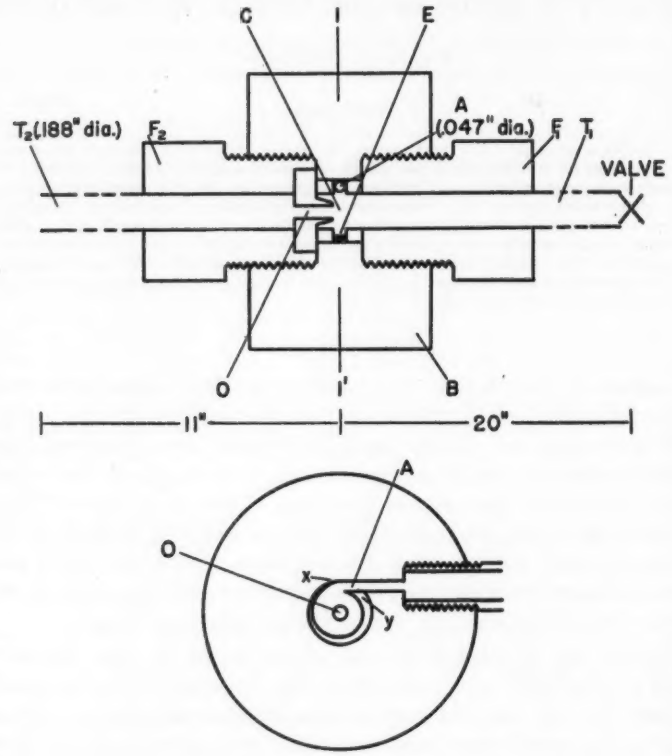

Fig. 1. *Cross section of heat separator.*

Gas is supplied at pressures from 30 to 200 p.s.i. (gauge), and escapes into the vortex chamber with a high tangential velocity. That portion of the gas that escapes through the small orifice (that is, from the centre of the vortex) is cooled, and that which escapes through the other end is warmed. The corresponding tubes will be referred to as the cold and warm ends respectively. The valve on the warm end is approximately 20 in. from the centre of the block, and controls the ratio of the flows through the two ends.

Measurements

The dimensions of the apparatus used for these measurements are approximately the same as those of the smallest size described by Hilsch. The temperatures of the two gas streams were measured by thermocouples at the following points: soldered to the warm tube near the valve; inserted into the

cold end so that the junction was close to the outer end of the orifice. The other junctions of both thermocouples were soldered to the inlet pipe near the block, *B*. These thermocouples could be read to a precision of 0.3° to 0.1° C. depending on the series resistance used.

In order to test the assumption, upon which Hilsch (1) has expressed doubt, that thermocouples so placed measure the temperatures of the gas streams, the enthalpy was calculated in selected cases. For example, with an input pressure of 90 p.s.i., input temperature of 17.2° C., and flow ratio (warm end flow to cold end flow) of 5.12, the warm and cold outlet temperatures were 23.3° C. and −19.8° C. respectively. With these data, the sum of the enthalpies of the exit streams agrees with that of the input within one per cent.

In order to determine the optimum working conditions, three variables were studied: input pressure, ratio of flows, and diameter of the orifice, *O*.

Using an orifice of 0.064 in. diameter, a series of measurements was made varying input pressure and flow ratio. These are in general agreement with graphs given by Hilsch. The maximum temperature drop observed was 42.8 centigrade degrees with input pressure, 200 p.s.i. and flow ratio, 4.3. However, this maximum temperature drop is obtained at the expense of a very low efficiency.

It is more significant to consider (i) the product of the temperature drop and the fraction of flow out the cold end, described below as 'useful cooling'; (ii) the ratio of the useful cooling to the theoretical adiabatic temperature drop at the same input pressure. The latter gives an indication of the efficiency as compared with an ideal expansion engine. The maximum useful cooling with this orifice was 10.6 centigrade degrees and the maximum efficiency was .071.

The graphs published by Hilsch indicate that the temperature drop at the cold end disappeared when the warm end flow was reduced to zero. In the author's experiments a cooling of 5.5 degrees was found when the warm end was closed, with an input pressure of 100 p.s.i. It is of interest that this cooling is definitely greater than that which the Joule–Thomson effect alone would produce, which for the above conditions is calculated to be 1.7 degrees.

Results of varying the diameter of the orifice, *O*, are also in general agreement with those of Hilsch. An orifice of 0.107 in. diameter was found to be the optimum in respect to both temperature drop and efficiency. This gave an optimum working condition at 100 p.s.i. as follows: flow ratio, 0.38; temperature drop, 24.8 degrees; useful cooling, 18.0 degrees; and efficiency, 0.14.

Samples of air taken from the two ends were analysed and the results gave no indication of any separation of the air into its components in passing through the heat separator.

A few measurements were made with carbon dioxide and hydrogen in place of air. For these gases a cold end orifice of 0.094 in. diameter was used, and the input pressure was kept constant at 100 p.s.i. These measurements are

summarized in the table below. The setting of the warm end valve was such as to give a flow ratio with air of 0.61. It was not possible to measure directly, with the rotameters available, the flow ratio for hydrogen. The figures for useful cooling and efficiency in this case are based on the assumption that the flow ratio is the same as that for air. The total flow through the apparatus is given in litres per minute at 1 atm. and 21° C. In the last column the product, Q_r, of the mass flow per minute through the cold end, the specific heat, and the temperature drop is given for each case. This product is a measure in calories per minute of the heat removed from the gas flowing out the cold end.

	Temperature drop, centigrade degrees	Useful cooling, centigrade degrees	Efficiency	Total flow, litres/min.	Q_r calories per minute
Air	19.5	12.1	.093	95	352
CO_2	19.8	12.3	.095	67	329
H_2	15.9	9.9	.076	430	1320

Possible Uses

It seems unlikely that the heat separator will replace any of the standard methods of refrigeration or gas liquefaction. It is less efficient than an expansion engine for liquefiers of the Claude type. Although it is more efficient than the Joule–Thomson effect for a liquefier of the Hampson type, the problem of removing the warm end flow without affecting the performance of the heat exchangers may make it impracticable. However, it is a simple and inexpensive device and can be a useful refrigerating agent for laboratory and other purposes where compressed air supplies are available and where more elaborate refrigeration machinery might not be economical.

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